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Influence of disseminated neoplasia, trematode infections and gametogenesis on surfacing and mortality in the cockle *Cerastoderma edule*

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ABSTRACT: *Cerastoderma edule* is a widely distributed bivalve mollusc, commercially exploited throughout Europe and is also an important food source for birds and crustaceans. Recently, mass surfacing and mortalities of cockles have been observed and reported at sites in Ireland and elsewhere, particularly in the summer months. One such site is Flaxfort Strand, Courtmacsherry Bay, County Cork, Ireland, an important feeding area used by many seabirds during the summer months. For the past few years large numbers of surfaced cockles have been observed at the site in a moribund condition. Samples of cockles from this area were collected over the summer months and their health status assessed. Cockles that had surfaced (moribund) and those still buried in the sediment were quantified and screened: sex, gonadal maturity and size class of cockles were also determined. Disseminated neoplasia and trematodes were observed in screened cockles. The most significant finding during the study was that mortalities and surfacing of cockles was related to a greater incidence of disseminated neoplasia. No neoplasia was observed in the smallest and largest size classes. There was a significantly higher prevalence of neoplasia in moribund cockles than in buried cockles, whereas in both groups a similar concentration of trematode metacercariae was observed in the screened tissues. Also, most of the cockles that had surfaced were either in the process of spawning or were spent. Overall a much larger percentage of moribund cockles exhibited both trematode infections plus neoplasia compared with buried cockles. A combination of the presence of neoplasia and trematodes, along with stress related to spawning, may immunocompromise the cockless, causing the animals to surface and become moribund.

KEY WORDS: *Cerastoderma edule* · Disseminated neoplasia · Trematodes · Surfacing · Gametogenesis

INTRODUCTION

The edible cockle *Cerastoderma edule* is a widely distributed bivalve mollusc in the family Cardiidae (de Montaudouin 1997, van der Veer et al. 2006). *C. edule* is a common infaunal species found in sandy bays and estuaries around Europe and often reaches high densities, e.g. thousands of adult cockles per square metre has been recorded in the Wadden Sea (Wegeberg & Jensen 2003). This cockle can tolerate air exposure, temperatures ranging from 3 to 25°C, salinities ranging from 10 to 35 and variable tidal strengths and can burrow to depths of 50 mm (Reise 2003). It is an active suspension feeder, subsisting on plankton and organic particulate matter (Kang et al. 1999, Strasser et al. 2001, Lehane & Davenport 2002, Rueda et al. 2005).

Cerastoderma edule is commercially fished in The Netherlands, France, the UK and Ireland with bottom trawls and dredges, and the largest catch is in The Netherlands (Seed & Brown 1977, de Montaudouin 1996, Piersma et al. 2001). The capture fishery for

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cockles peaked in 1987 when 106 494 tonnes of cockles were harvested, but decreased to 11 809 tonnes in 2009, the last year for which statistics are available (www.fao.org/fishery/species/3535/en; accessed 6 Sep 2011). Declines in yields can be attributed, in part, to the ban of mechanical cockle fishing in the Wadden Sea (The Netherlands) since 1 January 2005, and to further restrictions on other sand flats such as the Oosterschelde (www.seaonscreen.org/vleet/content/eng/cockle-fisheries.htm). Yields for cultured *C. edule* have been variable in the past (www.fao.org/fishery/species/3535/en; accessed 6 Sep 2011).

Sexual maturity is usually reached when cockles are 18 mo old or when shell length reaches 15 to 20 mm (Seed & Brown 1977). Seed & Brown (1977) reported that cockles overwinter in a spent condition, but gametogenesis from late autumn/early winter has also been described (Twomey 1987). Gonadal development occurs rapidly once initiated in the spring and spawning begins in late spring/early summer. *Cerastoderma edule* is a broadcast spawner; both sexes produce gametes that are released into the water column and fertilisation occurs externally (André et al. 1999). Planktonic larvae remain in the water column for 2 to 3 wk (de Montaudouin 1997, Bouma et al. 2001, Reise 2003) and experience rapid growth owing to high algal concentrations and increasing water temperatures (Honkoop & van der Meer 1998).

Previous studies have noted mass mortalities of cockles in several locations throughout Europe and large losses have occurred in recent years in some locations, such as the Burry Inlet in Wales, resulting in significant economic losses (DEFRA 2011). For many recent mortality events, a number of causative factors have been identified, including the presence of a number of haplosporidians in the Burry Inlet (ICES 2010, DEFRA 2011), trematode infections and the presence of disseminated neoplasia (Longshaw 2009). Digenean parasites were recorded during mortality events in Galicia, Spain, in 1997 (Carballal et al. 2001) and the northern Wadden Sea in 2004 (Thieltges 2006). In addition to trematode infections, cockle populations that experienced mortalities in Galicia also experienced disseminated neoplasia, which was considered to be another significant factor in contributing to the mortalities (Carballal et al. 2001, Villalba et al. 2001, da Silva et al. 2005). Although mass mortalities were described as having occurred, these losses were not quantified, as enumerating mass mortalities in burrowing animals in their natural habitat is difficult.

Of the various diseases and parasites that affect cockles, disseminated neoplasia and metazoan parasites have been reported most frequently in studies of *Cerastoderma edule* (Carballal et al. 2001, Villalba et al. 2001, Thieltges 2008, de Montaudouin et al. 2009). Neoplasms in *C. edule* were initially recorded in 1982 in Cork Harbour, Ireland, and in that same year they were also reported in Brittany, France (Twomey & Mulcahy 1988, Twomey 1994, Villalba et al. 2001, Romalde et al. 2007, Le Grand et al. 2010). Disseminated neoplasia is a proliferative disorder of the haemocytes and is of unknown origin; it is characterised by very large, mitotically active, pleomorphic and highly invasive cells (Twomey 1994, Krishnakumar et al. 1999, Collins & Mulcahy 2003, Barber 2004, Ciocan et al. 2006, Le Grand et al. 2010, Díaz et al. 2011).

Throughout their range *Cerastoderma edule* are known to act as the first or second intermediate host for at least 16 digenean parasites from 7 different families, of which the family Echinostomatidae is the most dominant (de Montaudouin et al. 2009). A recent study along the southwest coast of Ireland found 8 species of digenean parasites in *C. edule*, and *Meiogymnophallus minutus* (synonym *Parvatrema minutus*, Scholz 2002) was the most common in terms of prevalence and intensity of infection (Fermer et al. 2009). Of particular interest is the fact that trematode species contained within the cockle are restricted to certain tissues, so that a microhabitat is created within the cockle (de Montaudouin et al. 2009). Of those identified to date in Ireland, *M. minutus* is confined to the hinge region within the cockle, whereas *Gymnophallus choledochus* and *Bucephalus minimus* are located in the gonads. Others, such as *Psilostomum brevicolle*, are confined to the visceral mass only, while *Renicola roscovita* establishes itself in the palps, and *Himasthla elongata* and *H. interrupta* are located in the foot, although the latter trematode is also found in the mantle (de Montaudouin et al. 2009). *C. edule* may host such a range of parasites because its geographic range is extensive, the cockles may exist in close proximity to other intermediate hosts and the definitive hosts of these parasites also span a large area (Thieltges & Reise 2006, de Montaudouin & Lanceleur 2011).

The compartmentalisation of these trematodes needs to be taken into consideration when they are screened by means of conventional techniques. For example, with histological examination only a thin tissue section is screened, which undoubtedly will result in the underestimation of the total number of trematodes. Surveys of only trematode infections generally depend on full tissue squashing and screening to estimate total numbers (de Montaudouin et al. 2000). This method has limitations, however, as it does not allow the tissues to be screened to determine what other conditions or pathologies are present. So for cockles, in particular, both histology and screening of tissue squashes have their limitations owing to the complexity of conditions found in this particular bivalve. We chose histological examination as the method of screening to investigate all conditions, including reproductive development; but remained cognisant of the limitations of this technique as outlined above.

The aims of the present study were to investigate *Cerastoderma edule* surfacing events in a site designated as a Special Area of Conservation, and to attempt to quantify 'unusual mortalities' and determine what factors may be contributing to these mortalities. The health status of cockles was also compared by considering a range of parameters that included position (in the sediment or on the surface), sex and size class.

MATERIALS AND METHODS

Study site

The study was undertaken at Flaxfort Strand, Courtmacsherry, County Cork (51° 38′ N, 8° 41′ W) on the southwest coast of Ireland. Flaxfort Strand is a semi-exposed bay composed of extensive sand flats at low tide. There is freshwater input from a stream at the mouth of the bay and the area is surrounded by farmland. Throughout the course of the study there was an increasing proliferation of algal mats composed predominantly of *Enteromorpha* spp. The water quality in the area is classed by the Environmental Protection Agency as 'sufficient' (www.bathingwater.ie/epa/history.htm; accessed 20 Apr 2011). Courtmacsherry Bay is a Special Area of Conservation (SAC: 001230) as it is composed of 10 habitats recorded on Annex 1 of the European Union (EU) Habitats Directive (www.npws.ie/en/SAC/001230/; accessed 20 Apr 2011); nearby Clonakilty Bay is classed as a Special Protected Area (SPA: 004081) and also contains habitats classed as Annex 1 on the EU Habitats Directive (www.npws.ie/en/SPA/004081/; accessed 20 Apr 2011). Additionally, bar-tailed godwits *Limosa lapponica* and golden plovers *Pluvialis apricaria*, both of which are registered as Annex 1 on the EU Birds Directive, use this area (www.bathingwater.ie/epa/history.htm; accessed 20 Apr 2011).

Sampling of cockles

Fieldwork was performed at fortnightly intervals on 8 May, 27 May, 18 June, 5 July, 26 July and 23 August 2009 to reflect the time when most mortalities were occurring. A 400 m^2 area was delineated on the upper shore at the study site; its exact positioning varied only by a few metres on each sampling date. Cockles were taken from 2 positions: (1) 'moribund', i.e. live animals, plus clean empty shells, considered to be recently 'dead', on top of the sediment from the surface of the delineated area and (2) 'buried' live animals within the sediment (for sample sizes see Table 1). In order to subsample the cockles within the study area, random stratified sampling was carried out; a 0.25 m^2 quadrat was placed on the sediment 3 times based on coordinates generated by random number tables. Sediment was taken from the surface to the anoxic layer (approximately 12 to 15 cm in depth), and all live cockles within it were collected. In the laboratory, 30 moribund and 30 buried cockles were opened carefully (except for those in May 2009, see Table 1) and a transverse section of each cockle, through the digestive gland, gonads, gills, mantle, connective tissue and the tip of the foot, was preserved in Davidson's solution for histological examination (Shaw & Battle 1957, Howard et al. 2004) and refrigerated for 2 d at 4°C. They were then processed, embedded in paraffin, sectioned at 5 µm thickness, mounted on microscope slides and stained with haematoxylin and eosin.

Examination of slides

One slide per cockle was examined at 4×, 10× and 40× magnifications. All tissues were screened for prevalence and intensity of trematodes and incidence of disseminated neoplasia. For trematodes, since the whole animal was not screened as per de Montaudouin et al. (2009), a total number of trema-

Table 1. *Cerastoderma edule.* Numbers of cockles collected per sample and numbers screened by histological examination (in brackets) from May to August in 2009

Sample date	Recently dead	Moribund	Buried
8 May	281	49 (49)	46 (46)
27 May	186	23 (23)	24 (24)
18 Jun	534	128 (30)	88 (30)
5 Jul	666	267 (30)	60 (30)
26 Jul	443	98 (30)	36 (30)
23 Aug	477	129 (30)	60 (30)

todes per cockle could not be determined. However, as a transverse section from the same area of the cockle was taken for each individual, relative numbers of trematodes could be compared between cockles, and the prevalence and intensity of infection was based on the tissue section screened.

Reproductive stages were assigned to each of the cockles by using a modified scale as outlined in Drummond et al. (2006) and were denoted as inactive, early/late developing, ripe, spawning/partially spent and spent/resorbing. Occasionally, cockles presented stages that could be described as intermediate between 2 stages; in these cases the stage that dominated the greatest part of the gonads was assigned.

Statistical analysis

The data were analysed with the software SPSS 12.0.1 (for Microsoft Windows). A Kolmogorov-Smirnov nonparametric test was used initially to check the normality of the data. Independent samples *t*-tests were used to determine the significance of the prevalence and intensity of trematode metacercariae between surfaced and buried individuals. A Kruskal-Wallis test was used to ascertain whether the prevalence and intensity of metacercariae per size class were significantly different. A homogeneity χ^2 test was used to establish whether incidences of parasites and pathological conditions were significantly greater depending on sex or sample.

RESULTS

The density of surfaced cockles (dead and moribund) ranged from 6.5 to 29.1 cockles m^{-2} and the density of buried cockles varied from 32.0 to 117.3 cockles m^{-2} during the study period.

Morphometrics

The mean length ± SD of dead cockles increased over the duration of the study from 17.9 ± 4.9 to 21.4 ± 4.3 mm. Most cockles collected from the surface (dead and moribund) were in the 18.0 to 20.9 mm size class (29.3 % of dead cockles and 26.6 % of moribund cockles); however, shells collected from the surface varied from 4.2 to 37.3 mm. Most buried live cockles were in the 21.0 to 23.9 mm size class (25.8 % of cockles), although cockles ranged from 5.2 to 34.2 mm.

Sex ratio and gametogenesis

Of the cockles that could be sexed, there were a greater number of males collected during the study (130 males, 118 females). Of the moribund cockles, 50.9 % of cockles were male and 49.0 % were female; whereas, 53.4 % of buried cockles were male and 46.5 % were female. However, the proportion of males and females collected overall was not significantly different (χ^2 test, p = 0.4069).

Three stages of gonadal maturity were identified during the course of the study: ripe, spawning/partially spent and spent/resorbing. Moribund and buried cockles were not in synchrony in terms of gonadal maturity during the study (Fig. 1). Male moribund cockles (Fig. 1a) were either ripe (40.0 %) or spawning/partially spent (60.0 %) from the beginning of May, but buried cockles were observed at all 3 stages at the same time (ripe, 30.7 %; spawning/partially spent, 30.7 %; spent/resorbing, 38.6 %). Moribund males could be identified again in late May, but owing to neoplasia and trematode infection the gonads were in poor condition and a stage could not be accurately assigned; this was common among male and female moribund cockles; hence, no males were observed in 27 May 2009 samples (Fig. 1a). There was also evidence for 2 spawning events, but the timing for these events differed for moribund and buried cockles. Male moribund cockles were not recorded as spent/resorbing until June and were seen in ripe condition again from early July. Females, both moribund and buried, were in ripe condition from early May, but buried cockles were already spawning/partially spent (53.8 %), and by mid-May all cockles were spawning/partially spent (Fig. 1b). Moribund female cockles were again seen in ripe condition from June, but buried female cockles were not in ripe condition until early July. By August all buried female cockles were spent/resorbing (Fig. 1d), but some moribund cockles were still spawning/partially spent (15.3 %).

Disseminated neoplasia

Two types of neoplastic cells were identified. The most common cells were lightly stained and showed discernible cell contents Those of the second type were darkly stained as previously described by Twomey (1987) and Carballal et al. (2001) (Fig. 2b). Both neoplastic cell types were seen together in approximately 25.0 % of all cockles with neoplasia. In extreme cases neoplastic cells infiltrated and obscured normal tissues (Fig. 2a).

The mean prevalence ± SD of neoplasia among all cockles at Flaxfort Strand during the study period was 21.8 ± 9.6% and ranged from 15.0 to 38.3%. Overall, in moribund cockles the mean prevalence of neoplasia was 35.6 ± 16.0%, while among buried cockles it was 6.0 ± 4.0%. The prevalence of neoplasia among surfaced cockles was consistently greater on each date sampled and this was significantly dif-

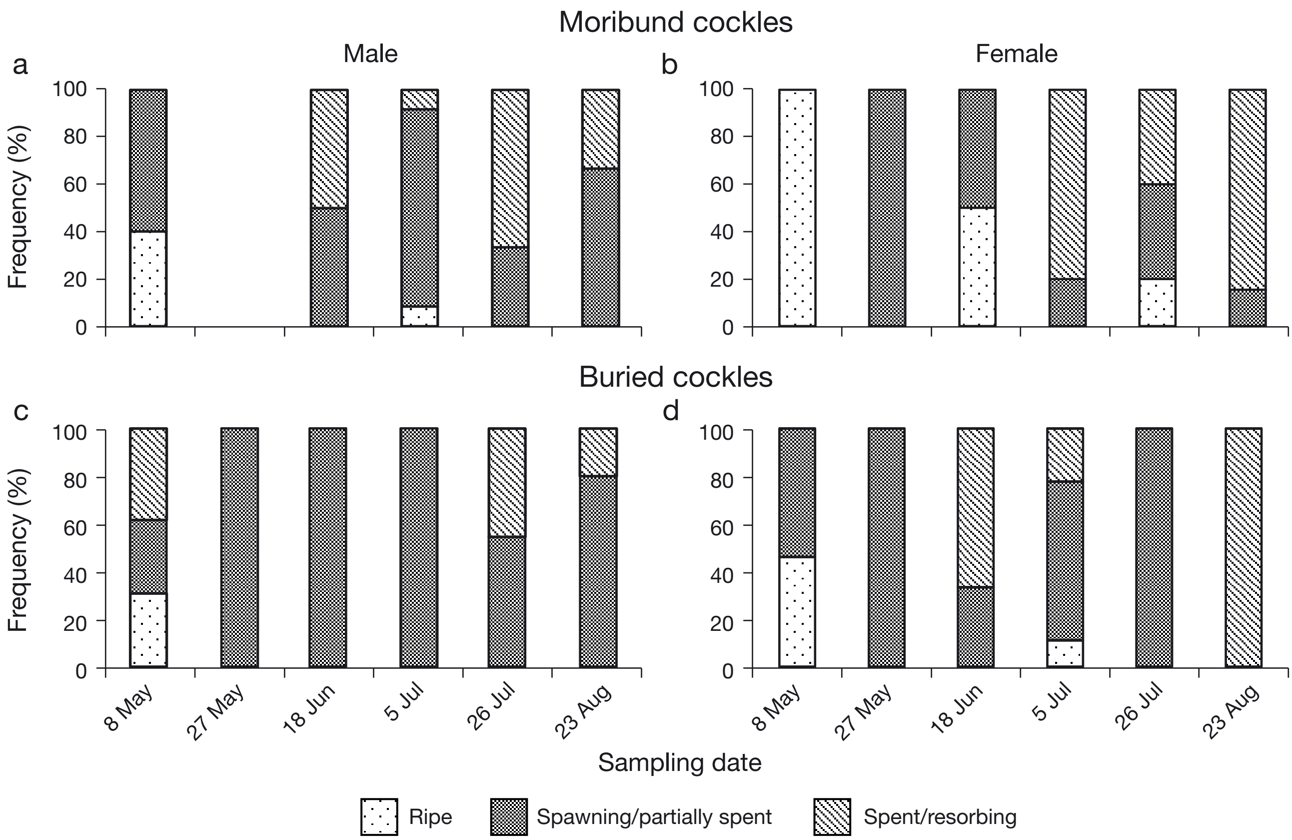

Fig. 1. *Cerastoderma edule*. Percentage of moribund (a) male and (b) female cockles and buried (c) male and (d) female cockles at each of the 3 stages of gonadal development: ripe, spawning/partially spent and spent/resorbing. Samples were taken from May to August 2009

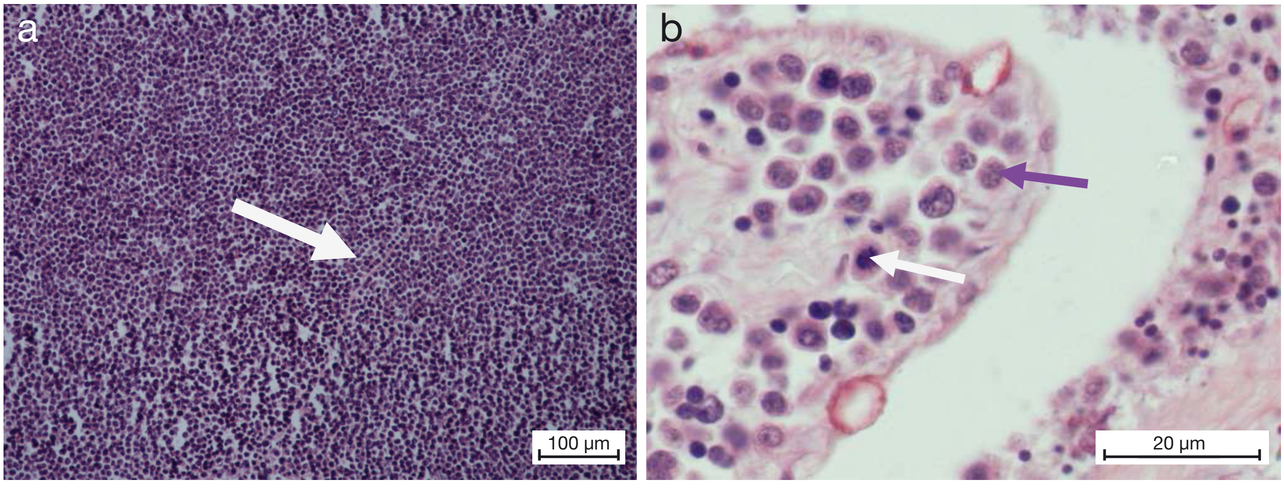

Fig. 2. *Cerastoderma edule*. Histological sections showing disseminated neoplasia in the cockle (H & E stain). (a) Extreme proliferation of neoplastic cells with only a small amount of original tissue remaining (arrow). (b) Section shows both kinds of neoplastic cells: darkly stained (white arrow) and lightly stained with discernible cell contents (purple arrow)

ferent from that in buried cockles (Mann-Whitney test, $p < 0.05$) (Fig. 3). When all cockles from both subsamples were pooled (moribund and buried) neoplasia occurred most often (23.1%) in intermediately sized cockles (18.0 to 20.9 mm), but it was seen in cockles ranging in size from 12.5 to 32.9 mm. It was absent in the smallest (9.0 to 11.9 mm, n = 8) and largest (33.0 to 35.9 mm, n = 1) cockle size ranges; however, the number of cockles in these size classes was low (Fig. 4). Although neoplasia was seen most often in female cockles (14.4% of females compared with 8.4% of males) there was no significant difference in the prevalence of neoplasia among male and female cockles when moribund and buried cockles were combined (χ^2 test, p = 1.32).

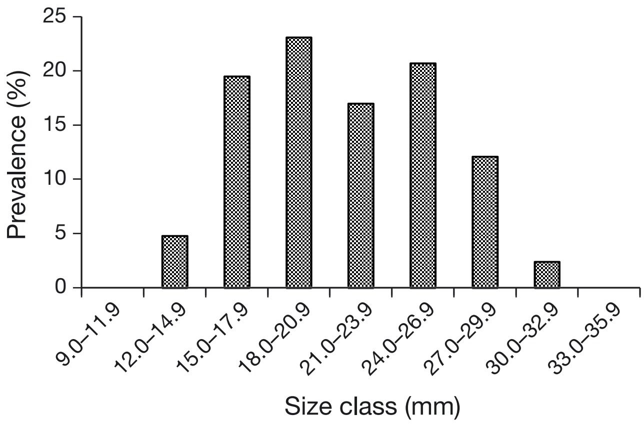

Fig. 4. *Cerastoderma edule*. Prevalence (%) of neoplasia in screened cockles of different size classes (mm)

Trematode infections

Trematodes were most commonly encountered in the gonads and the intestinal lumen. They attained high numbers in both areas and haemocyte proliferation could occasionally be observed in individual cockle tissues surrounding the parasite. Owing to their location within the tissues and based on previous studies performed at this site (e.g. Fermer et al. 2011) the trematodes were identified as *Meiogymnophallus minutus*, *Gymnophallus choledochus*, *Bucephalus minimus* and *Psilostomum brevicolle.* For this study parasitised individuals were divided into those infected with sporocysts only (first intermediate host: *B. minimus*), those infected with both sporocysts and metacercariae (first and second intermediate hosts: *B. minimus* and *G. choledochus*) and those containing metacercariae only (second intermediate hosts: *M. minutus* and *P. brevicolle*).

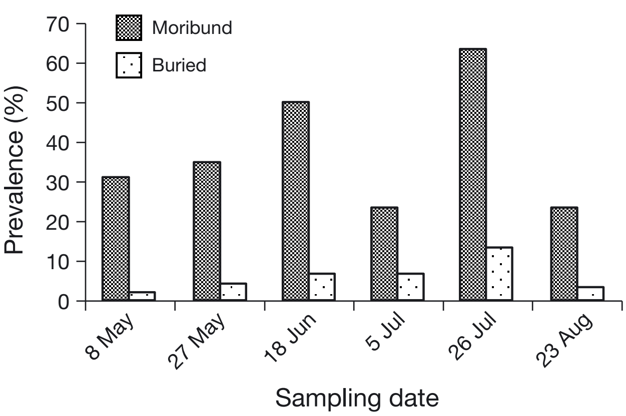

Fig. 3. *Cerastoderma edule*. Prevalence (%) of neoplasia in moribund and buried cockles collected during the study period from May to August 2009

Cockles acting as a first intermediate host and infected with sporocysts only were recorded on 26 July 2009 (Fig. 5a) and had a prevalence of 10.0% (3 of 30 moribund cockles) and an intensity of 46.6 ± 79.1 sporocysts. Sporocysts and metacercariae were noted on 27 May 2009 in one buried cockle (prevalence of 2.1%, intensity of 11 metacercariae and 99 sporocysts) and in one cockle (moribund) on 5 July 2009 (prevalence of 1.6%, intensity of 87 sporocysts and 3 metacercariae) (Fig. 5b). Metacercariae in isolation were far more prevalent; the mean (combined moribund and buried) prevalence was 38.4 ± 22.2%, There was a greater mean prevalence of metacercariae in buried cockles (39.3 ± 22.6%) than in moribund cockles (35.3 ± 25.9%), but the difference was not significant (Fig. 5c). The mean overall intensity was 13.7 ± 6.1 metacercariae per cockle; there was no significant difference between moribund and surfaced cockles, although metacercariae intensity was slightly higher among moribund (15.1 ± 3.4 metacercariae per cockle) than buried cockles (13.1 ± 7.8 metacercariae per cockle) (Fig. 6).

Trematodes were not found in the smallest cockle size range (9.0 to 11.9 mm) and their prevalence was low in cockles longer than 30.0 mm (for cockles 30.0 to 32.9 mm the prevalence of trematodes was 1.3%; for cockles 33.0 to 35.9 mm the prevalence of trematodes was 0.7%). Trematodes were most commonly recorded in the 21.0 to 23.9 mm size class, followed by 18.0 to 20.9 mm (Fig. 7). Trematode intensity and prevalence per size class did not differ significantly.

Of the 113 cockles of known sex and parasitised by metacercariae only, 47.7% were male and 52.2% were female (cockles of indeterminate sex were also parasitised). There was no significant difference in

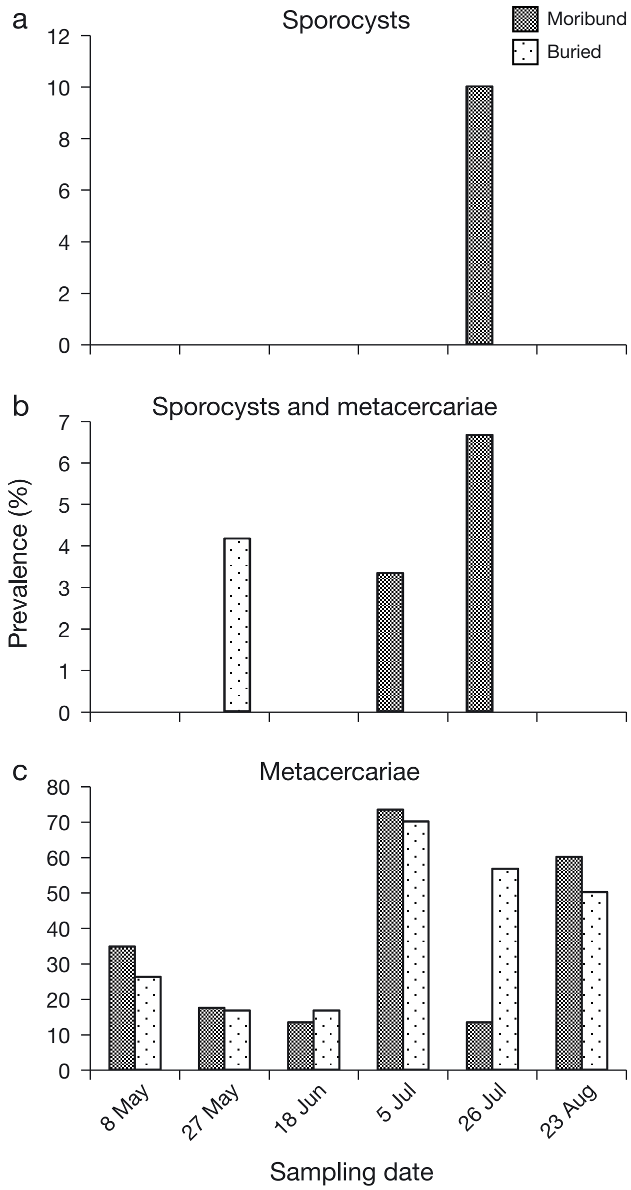

Fig. 5. *Cerastoderma edule*. Prevalence (%) of trematode (a) sporocysts, (b) sporocysts and metacercariae and (c) metacercariae only in moribund and buried samples of cockles collected from 8 May to 23 August 2009

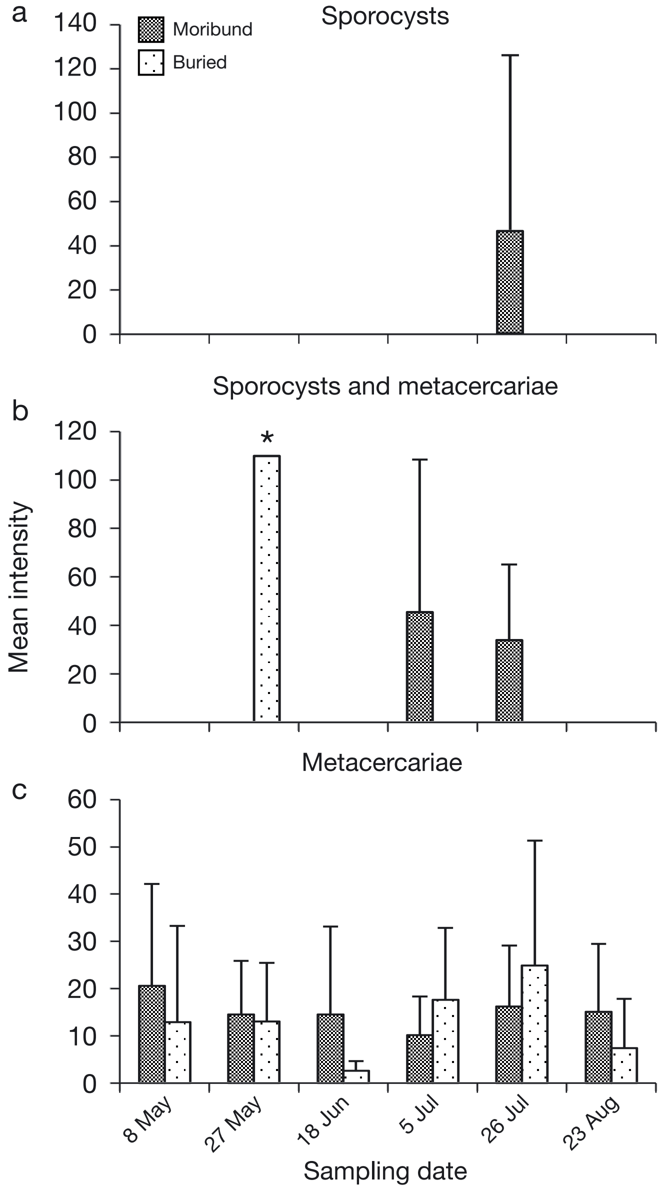

Fig. 6. *Cerastoderma edule*. Mean intensity ± SD of trematode (a) sporocysts, (b) sporocysts and metacercariae and (c) metacercariae only observed in the screened tissue sections of moribund and buried cockles during the study period. Asterisk (*) indicates only 1 cockle was infected with sporocysts

the numbers of male and female cockles parasitised throughout the study when all cockles both moribund and buried were pooled (χ^2 test, p = 0.06)

Combination of conditions

Trematodes alone (metacercariae and sporocysts) were only found in 26.0% of all moribund cockles (n = 192) and in 36.9% of buried cockles (n = 190). Trematode mean intensity was marginally greater in buried (18.7 ± 12.7 trematodes per section/cockle) than in moribund (17.5 ± 5.6 trematodes) cockles. However, trematodes were observed in conjunction with neoplasia in 14.6% of moribund cockles in which the mean trematode intensity was 22.9 ± 29.2 trematodes, compared with 3.1% of buried cockles in which the trematode intensity was 33.2 ± 28.9 trematodes. These observed values of combined trematode and neoplasia infections differ from the expected values calculated with an equation modified from Sannia & James (1978):

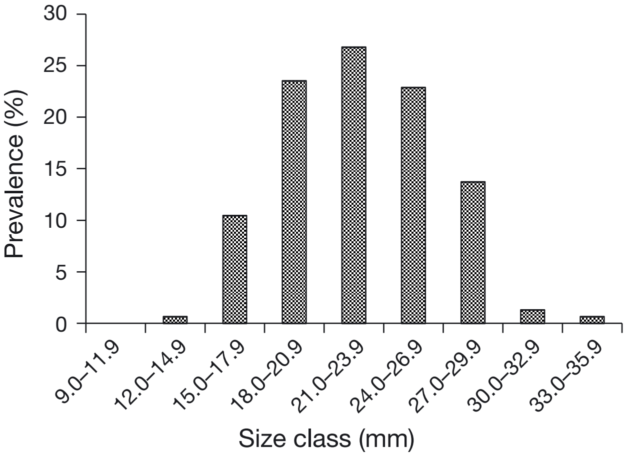

Fig. 7. *Cerastoderma edule*. Prevalence (%) of all trematodes combined observed per cockle size class during the sampling period

$$\text{Expected dual infection} = \frac{P_{Trem} \times P_{Neopl}}{100}$$

where P_{Trem} = observed trematode infection prevalence and P_{Neopl} = observed neoplasia prevalence.

Expected dual infection among moribund cockles was 5.8%, but 14.6% was actually observed; similarly expected dual infection in buried cockles was 1.0%, but it was observed in 3.1% of the animals. Significantly higher levels of dual infections were seen in moribund cockles than in buried cockles (χ^2 test). Of buried cockles 2.6% had neoplasia only, whereas 22.4% of moribund cockles were identified with neoplasia only. Of moribund cockles 37.0% had neither neoplasia nor trematode infection, and among buried cockles this number rose to 57.4% (Table 2).

DISCUSSION

Assessing mortality levels and surfacing events in wild cockle fisheries can be difficult owing to a number of factors, including movements of animals because of tides, predation and time spent on the surface.

Disseminated neoplasia was the most significant factor that distinguished the surfaced and buried cockles, which is similar to that found in other studies (Le Grand et al. 2010), and this supports the hypothesis that disseminated neoplasia could influence surfacing of cockles. Cockles were also infected by digenean trematodes, but the contribution of these parasites was less clear; however, a much larger percentage of surfaced cockles had trematode infections plus neoplasia compared with buried cockles.

Prevalence of neoplasia reached as high as 84% among populations of *Cerastoderma edule* showing mortalities in areas of northwestern Spain in June 1997 compared with 4% prevalence in nonaffected populations (Villalba et al. 2001, Barber 2004).

Mortalities of *Cerastoderma edule* juveniles determined by using cohort monitoring may range from 56 to 100% (Gosselin & Qian 1997). The complexity of determining actual mortality levels may explain why mortalities in many studies are referred to but not quantified. A number of studies refer to 'high' mortalities (e.g. Villalba et al. 2001, Carballal et al. 2001, Thieltges 2006), but they are not quantified. Some controlled studies have been carried out for cockles (Blanchet et al. 2003) and other bivalves, e.g. *Mya arenaria* (Barber 2004). Exact quantification of mortality in bivalves would require some form of containment above the sediment to determine mortality rates during the study and initial numbers of cockles would also need to be known as outlined in Twomey (1987), who determined mortality of experimentally infected cockles as well as control cockles in a caged experiment.

There was a significant difference in the mean lengths of cockles collected per subsample. Dead cockles were significantly smaller than moribund and buried cockles. Mortality in this size class may

Table 2. *Cerastoderma edule*. Prevalence of disseminated neoplasia and trematode infection and mean (±SD) intensity of trematodes (number per section/cockle) in uninfected cockles, cockles diagnosed with trematode infection only, cockles with disseminated neoplasia only and cockles infected with both trematodes and neoplasia together when all trematode reproductive stages were grouped

Condition	Moribund cockles		Buried cockles	
	Prevalence (%)	Trematode intensity	Prevalence (%)	Trematode intensity
Uninfected	36.97		57.36	
Trematode infection only	26.04	15.5 ± 18.13	36.84	15.7 ± 18.1
Disseminated neoplasia only	22.39		2.63	
Trematode infection and disseminated neoplasia	14.58	22.9 ± 29.19	3.15	33.2 ± 28.89

have been greater as neoplasia and trematodes were most prevalent in the same size class (18 to 20.9 mm). When all of the cockles were combined the prevalence of neoplasia was greatest in cockles of this intermediate length. Sexual maturity in cockles is attained typically at 18 mo or at a size length of 15 to 20 mm (Seed & Brown 1977, 1978, van der Veer et al. 2006). The combination of neoplasia, parasitism and the stress of spawning for the first time may have caused greater mortality in this size range of cockles.

Densities of buried cockles were low in comparison with the upper limits known from other studies; for example, Fermer et al. (2011) described densities of 312.8 ± 81.0 individuals m^{-2} in the same area as the present study. In the present study there were a greater number of male than female cockles, but not significantly so. Reproductive maturity of male and female cockles was not always synchronous in either the moribund or buried cockles; however, Kingston (1974) has described coordinated spawning in cockles. In the present study it appeared that spawning occurred in small numbers of individuals throughout the study period. In contrast, Seed & Brown (1977) found in Strangford Lough that the majority of cockles spawn together at the start of the summer and the remainder can spawn later in the summer. Of interest in the present study was that moribund cockles appeared to be in the process of developing mature gonads and were spawning more rapidly than buried cockles; this precociousness may have exacerbated the stress in these individuals, causing them to surface.

Abnormal neoplastic cells identified in the study could be subdivided into 2 basic cell types, which is in agreement with other studies on neoplasms in bivalves (Twomey 1987, Carballal et al. 2001, da Silva et al. 2005, Díaz et al. 2011). According to Carballal et al. (2001) the different cell types could be representative of different stages of the condition's progression. In the present study, the degree of neoplasia was not quantified other than noting that the condition was present. In another study, Le Grand et al. (2010) classified the degree of neoplasia by means of 3 different techniques and scales: assessment of haemolymph cell monolayers, histological examination and flow cytometry of the DNA content in the cockle cells. Generally the condition was localised within the tissues and few cockles displayed the neoplastic condition throughout all of the tissues.

The prevalence of neoplasia has been in some cases linked to higher temperatures, such as in a previous Irish study (Twomey 1994); however, in a study of disseminated neoplasia in *Cerastoderma edule* from Arcachon Bay, France, prevalence of neoplasia was not related to water temperature (Le Grand et al. 2010). In the present study the highest prevalence of neoplasia among moribund and buried cockles was observed in July, so the influence of temperature on neoplasia development may be only one of a range of factors that are significant. The greatest prevalence of neoplasia in a previous study from the south of Ireland was reported in June and November (71 and 72 % prevalence at Cuskinny and Cobh, respectively, in County Cork) (Twomey & Mulcahy 1988). Disseminated neoplasia is thought to occur continuously in cockles, but the prevalence in a population has been shown to multiply greatly and rapidly and can vary hugely in successive years (Barber 2004).

Sex did not appear to influence the presence or absence of neoplasia. The prevalence of neoplasia was higher in females than in males during the course of the present study, but not significantly so. A previous study of *Cerastoderma edule* in Ireland by Twomey & Mulcahy (1988) suggested that disseminated neoplasia arises more often in females or in younger males among 11 harbour sites sampled and showed that 39 % of females and 26 % of males were diagnosed with disseminated neoplasia. Neoplastic cells proliferate rapidly and this may be energetically expensive, leaving fewer resources to donate to gametogenesis (Barber 2004), which, when combined with the stress associated with spawning in the cockles in the present study, may be another contributing factor to surfacing events. Neoplastic cells lose cell functions such as the ability to phagocytose; this may negatively affect their assimilation of nutrients in the animal (Barber 2004) and, in turn, result in some immunosuppression (Díaz et al. 2011).

Trematodes were observed in 40.4 % of the cockles examined. There were 2 different stages present, predominantly metacercariae and very occasionally sporocysts enclosing cercariae (sporocysts were found only in 3 cockles on 5 July 2009, and sporocysts in combination with metacercariae were at low prevalences in May and July). Sporocysts were always observed in very high numbers and their infiltration caused much destruction of the tissues. Fermer et al. (2011) also found metacercariae to be more common than sporocysts of parasites in *Cerastoderma edule* from the south of Ireland.

The greatest prevalence of trematodes (all reproductive stages) was in July. Shedding and transmission of trematode larvae is known to be temperature dependent (Jensen et al. 1999, Gam et al. 2008, Thieltges 2008). In temperate tidal areas the period of infection is spring to autumn (Desclaux et al. 2006, Thieltges 2008). Infectivity of cercariae is increased at higher temperatures until a certain threshold is reached (Jensen et al. 1999), which may explain the observations in the present study.

There was no significant difference in the prevalence or intensity of trematode metacercariae in surfaced or buried cockles, or per size class or among the sexes. Trematode infections have been found in cockles as small as 4 mm in a previous study of this site and nearby sites (Fermer et al. 2009). There was no significant difference in the intensity per size class, but the highest intensity was in the 12.0 to 14.9 mm size class in which only 1 cockle out of 21 was infected. The second highest intensity was in the 15.0 to 17.9 mm size class, which had a larger sample size (16 of 41 infected). Other studies have found greater intensities of trematodes in older, larger cockles that were related to the duration of exposure and infiltration rate of the trematodes, and there is experimental evidence of cockle siphons rejecting cercariae owing to the size of the cercariae (Jensen et al. 1999, Desclaux et al. 2004, 2006, de Montaudouin et al. 2005, Thieltges 2008). Shell size and accumulation of metacercariae may only matter when the cockles are smaller. Fermer et al. (2009) found the acquisition of metacercariae was associated with shell length when cockles were smaller, but this was less important in older cockles. However, others have found no relationship between size and intensity (Gam et al. 2008). The assumption that larger cockles would have a higher intensity of trematodes is based on larger cockles having larger siphons and ingesting cercariae with greater ease. But, it is known that there is great plasticity in cockle siphon length and cercariae may be of different lengths (de Montaudouin et al. 2005, Desclaux et al. 2006). The methodology used may also have partially influenced the results; a section was cut through each animal and all trematodes in that section were counted; therefore, the whole body was not screened and trematode numbers may have been underestimated. However, as all cockles were treated in exactly the same way, valid comparisons can be made between individuals.

Smaller cockles (<24 mm), especially those in the 18.0 to 20.9 mm size class, appear to have been differentially affected by the various factors in this study and were subject to greater mortalities. The mean length of dead cockles collected throughout the study was from 17.9 to 21.4 mm, and the greatest prevalence of neoplasia in all cockles gathered was in the 18.0 to 20.9 mm size range. Although the greatest prevalence of trematodes was found in the 21.0 to 23.9 mm size range (26.8%), the second greatest prevalence was in the 18.0 to 20.9 mm cockles (23.5%). Cockles become sexually mature at age 18 mo or at 15 to 20 mm (Seed & Brown 1977, 1978, van der Veer et al. 2006) and the stress of reproducing for the first time and the energy expended producing gonads may leave the cockles in a weakened, susceptible condition. It may therefore be easier for parasites to become established and for neoplasia to progress.

In conclusion, although neoplasia and trematodes were both diagnosed in cockles from Flaxfort Strand, neoplasia appears to have had a greater influence on mortality, as there was a significantly greater prevalence of neoplasia in surfaced than in buried cockles. Of interest was the large percentage of these organisms that displayed dual infections, i.e. neoplasia plus trematodes. The stress of spawning may have further immunocompromised the cockles, leading to surfacing and death. The long-term causes of these mortalities occurring at this site may be influenced and amplified by the presence of large algal mats and secondary opportunistic infections, factors that will require further examination in future studies.

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